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Partition Coefficients (Free Ligands and their Iron(III) Complexes) and Lipophilic Behavior of New Abiotic Chelators. Correlation to Biological Activity

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Abstract: Partition coefficients between n-octanol and water have been measured for ten tripodal ligands with catecholate or hydroxyquinolinate or pyridinophenolate chelating subunits and for their iron(III) complexes. The abilities of the ligands to cross an octanol phase and to extract ferric ion from its EDTA complex in an aqueous phase are studied. Correlation with biological properties are discussed.

Introduction

Siderophores are iron-chelating agents which are excreted by micro-organisms to render iron soluble in the environment and favour the uptake of this metal ; plants have developed different strategies for enhancing iron uptake, one of them using phytosiderophores.[1, 2] Iron chelation by some natural or abiotic chelators can be applied to human diseases characterized by iron overload. Water-soluble iron complexes of similar chelators can be used to alleviate iron deficiency in plants, preventing and even reversing iron chlorosis.[1, 2] The ability of the chelator and of its iron complex to fulfil these functions is primarily related to their powerful and selective affinity for the metal (revealed by the pFe value[3]). Water solubility is needed, but the access to the cell, through biological membranes, of the chelators or of their iron complexes, depends on their lipophilicities. This lipophilicity is conveniently measured by the partition coefficient P between n-octanol and water.[4] We have developped the efficient water-soluble chelator O-TRENSOX (Figure 1).[5-8] O-TRENSOX exhibits strong complexing ability for ferric iron, of the same order of magnitude as the tris catecholate (TRENCAMS).[9] Administred to rats which have been iron-loaded, O-TRENSOX is able to mobilize iron from hepatocytes and exerts protective effects on iron toxicity.[7] Ferric O-TRENSOX was found able to prevent and to reverse iron chlorosis in several plant species.[6] We have also developped C-derivatives of O-TRENSOX (the spacer in the tripodal backbone is a C-functionalized subunit instead TREN)[10] and a new ligand, TRENPYPOLS which incorporates three 2-(2-hydroxyphenyl) pyridine subunits and whose iron complex is efficient for the growth of *Arabidopsis thaliana*

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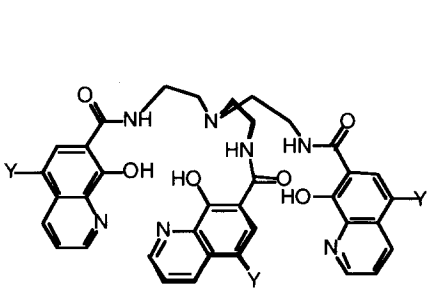
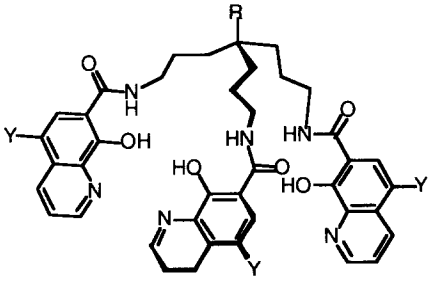
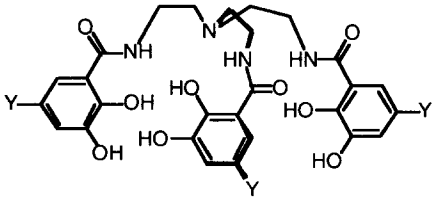
plant cells.[11] The ligands discussed in this paper are depicted in Figure 1. They all exhibit strong complexing ability toward ferric ions (≥ EDTA).



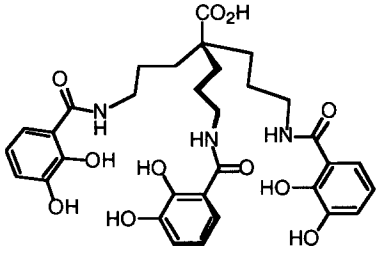
O-TRENOX (Y = H)
O-TRENSOX (Y = SO_3^-)



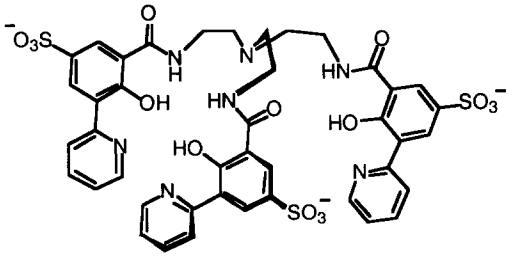
C_H (Y = SO_3^-, R = H)
C_{alc} (Y = H, R = CH_2OH)
C_{ac} (Y = H, R = CO_2H)
C_{est} (Y = H, R = CH_2O-$COCH_3$)



TRENCAM (Y = H)
TRENCAMS (Y = SO_3^-)



C_{ac}CAM



TRENPYPOLS

Figure 1: Iron chelators

The partition coefficients have been previously determined for chelators and for their Fe(III) complexes in the pyridoxal isonicotinoyl hydrazone class[12,13] and in the 3-hydroxy-4 (1H)-pyridinone class[14]. This paper

reports the partition coefficients of the chelators depicted in Fig.1 and for their iron(III) complexes. Experiments using a triphasic system mimicking a biological membrane are also described, allowing the comparison of the abilities of the various ligands to cross a lipophilic phase.

Results and discussion

Partition coefficients:

The results concerning the partition coefficients are given in Table 1.

Table 1 : Partition coefficient (*P*)*

LIGAND	*P* (% mol in water) free ligand	*P* (% mol in water) iron complex
O-TRENOX	10.1 (9)	(insoluble in both phases)
O-TRENSOX	0.02 (98)	< 0.005 (100)
TRENCAM	15.7 (6)	0.01 (99)
TRENCAMS	< 0.005 (100)	< 0.005 (100)
TRENPYPOLS	0.02 (98)	0.03 (97)
C_H	0.01 (99)	0.03 (97)
Cac	4.3 (19)	(insoluble in both phases)
Cest	24 (4)	(insoluble in both phases)
Calc	15.7 (6)	(insoluble in both phases)
CacCAM	3.2 (24)	0.01 (99)
EDTA	0.13 (88)	< 0.005 (100)

* defined by the concentrations (mol L^{-1}) ratio in the two solvents (octanol/water)

All the sulfonated ligands, which have been built to allow water-solubility, exhibit a partition coefficient close to zero, independently of the spacer or of the complexing subunits. The corresponding complexes, which bear an important electric charge exhibit the same behavior. In the absence of sulfonate groups, the C-CO_2H - substituted spacer confers water-solubility on the free ligand at a greater extent than do the other spacers. In the case of the unsulfonated ligands, only the iron complexes bearing an electric charge exhibit water-solubility (complexes from TRENCAM and CacCAM). A linear relationship is often used to correlate the partition coefficients of the free ligand and of the iron complex[12,13] ($\log P_{comp} = n \log P_{free\ lig} + k$) where n is the stoichiometry of the complex and k is a constant representing the change in hydrophobicity in converting the ligating groups of the ligand into the iron coordination sphere of the complex. The results of Table 1 show that

this relation cannot be used with highly hydrophilic ligands (sulfonated ligands). The accessibility to the solvent for the sulfonate groups, which are external to the coordination sphere, is the same in the free ligand and in the complex which remains highly hydrophilic. As previously claimed[13], the calculation of log P_{comp} from log$P_{free\ lig}$ by using the equation may lead to erroneous results ! None of the ligands exhibit solubility in the two phases, both in the free state and in the iron complex state. It is usually claimed that biological activity requires $P \approx 1$ (log$P \approx 0$) for the free ligand and for the complex. This has been recently corrected and log P values ≈ 3 were found to be the best values for chelators in the pyridoxal isonicotinoyl hydrazone class for mobilizing iron from reticulocytes[13]. Solubility of the iron complex is required to avoid precipitation. So, not one of the chelators depicted in Fig.1 is expected to exhibit a biological activity requiring to cross the cell membrane. Nevertheless, O-TRENSOX and TRENPYPOLS do it!

Water-octanol-water triphasic systems:

In order to mimick a transmembrane transport system, a triphasic system involving an octanolic phase in contact with two separated aqueous phases (buffered at pH=7) has been established (see Figure 3 in Experimental Part). The free ligand is in the aqueous phase A, which mimicks the external medium. The iron (III) complex of EDTA is located in the aqueous phase B, mimicking the chelated intracellular iron. The studied ligands are stronger complexing agents than EDTA and, moreover, the partition coefficient of Fe-EDTA is very low: therefore, the iron movement goes from B to A and the system is not leaky. The formation of the iron complexes with the ligand is monitored by UV-visible spectroscopy of the Fe-EDTA solution B. Only the sulfonated ligands O-TRENSOX, TRENPYPOLS, C_H and the unsulfonated TRENCAM and CacCAM can be studied. No change is observed with the sulfonated ligands: in accordance with the values of P, these ligands are unable to cross a lipophilic phase. Results concerning TRENCAM and CacCAM are depicted in Figure 2. The LMCT band of the ferric complex of the ligand appears in the EDTA-containing phase. The exchange of iron (which is complete in ten minutes in a simple aqueous medium) requires more than 150 hours to be achieved through the octanolic membrane (Figure 2b).

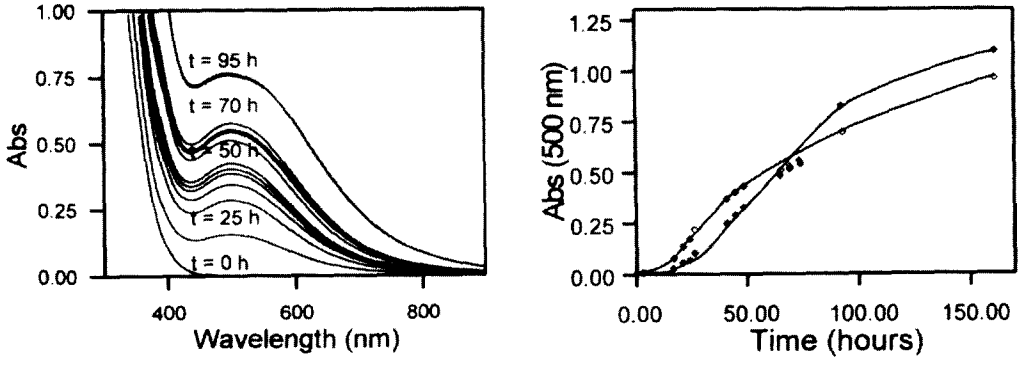


Figure 2: (a) UV-vis spectra (*vs* time) for the phase B ([Fe-EDTA] = 1mM) with CacCAM in phase A (0.8 mM); **(b)** Absorbance (at 500 nm) *vs* time for Fe-EDTA (0.8 mM) in B with 0.8 mM CacCAM (◊) or with 1 mM TRENCAM (•) in A. Tris HCl 0.05 M, pH 7; 25 °C.

The most interesting conclusion (correlated with the previous biological studies of TRENSOX, TRENPYPOLS and their iron complexes) is that *lipophilic solubility is not a necessary prerequisite for transmembrane iron transport*. This may reveal the occurrence of mechanisms not still elucidated. The nutritional iron uptake, which occurs from abiotic iron complexes in the outer part of the cell membrane and which takes place only when the cell is iron deficient, may be allowed by the "high iron chelating power of the cell" in this situation. The addition of a free ligand in the external medium can induce the reverse motion of the iron: the antiproliferative effects exhibited by O-TRENSOX may involve the capture of iron ions at the outer face of the membrane, lowering the iron status of the cell via removing equilibrium state. Of course, mechanisms more sophisticated than simple diffusion may occur. Further studies are required for the understanding of the "black boxes" revealed by our results.

Materials and methods

All reagents were of the finest quality available commercially. All solvents were distilled prior to use. The syntheses of the iron chelators and of their iron (III) complexes are described elsewhere. Electronic absorption spectra were recorded on a Perkin-Elmer Lambda 2 spectrometer using 1.0 cm pathlength quartz cells and connected to a IBM PC 340 microcomputer. The partition coefficients between an aqueous phase buffered at pH 7 (TRIS buffer) and octanol, were determined according to a known procedure.[15] The triphasic system is depicted in Figure 3 (2.5 ml of a TRIS-buffered at pH=7, 1 mM solution of the free ligand, 6 mL octanol, 2.5 ml of a buffered 0.8 mM solution of Fe-EDTA). The time-dependence of UV-visible spectrum of the Fe-EDTA solution is followed. Fe-EDTA being photoreducible, the experiments were performed in the dark.

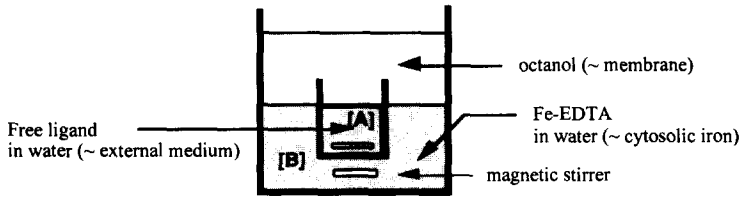


Figure 3: the triphasic system

Acknowledgments

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